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NUTRITION REVIEWS

VOL. 13 JULY 1955 No. 7

NUTRITIVE EFFECTS ON FOODS STERILIZED BY IONIZING RADIATIONS

Within the past decade considerable interest has been developed in the so-called "cold sterilization" of foods by ionizing radiations. Scientists have endeavored to utilize the bactericidal properties of ionizing radiations ever since the discovery of roentgen rays in 1895. Only in recent years, however, with the development of particle accelerators, nuclear reactors and the ultimate availability of mixed fission products has there been a concerted effort to harness this type of radiant energy to the processing of foods, pharmaceuticals, and biologic materials. The possibilities would appear unlimited and are most challenging to the food technologist.

For the preservation of foods and prevention of bacterial spoilage such methods as thermal treatment (canning of foods), dehydration and freezing have been practiced. Much interest was developed in World War II in the dehydration of foods because of the obvious advantages in keeping quality and saving in shipping space. While thermal treatment and freezing of foods, the more common current practices, are widely accepted methods, they have certain disadvantages. The major drawback in handling of frozen foods is the large requirement for refrigeration space and the cost of operation which are of paramount importance in military operations. Significant improvements have been made in the quality of thermally processed canned foods; however, there are a number of canned food items today, such as canned meat products, which require excessive amounts of heat energy for sterilization and consequently do not result in the attainment of the best flavor and texture. With the potential commercial development and utilization of radiation sterilization, it would be possible to conserve on space and labor requirement necessitated in the heat sterilization and canning of foods. Radiation sterilized foods packaged in thin aluminum or plastic containers would not only effect a saving in space, but would obviate the necessity for refrigeration, both important considerations in military transportation of preserved foods. In addition, the advantages of supplying sterile, stable and not overly processed foods such as vegetables, fruits and meats to front line troops and hospital patients are obvious. For civilian use the nonrefrigeration of foods processed by this newly developed method offers certain distinct advantages.

Whereas the major emphasis has been placed on the processing of foods by beta or gamma radiation, progress has also been made in the treatment of heat-sensitive pharmaceuticals used in large quantities today, such as the antibiotics. Consequently these products, being heat-labile and produced from microorganisms, present certain difficulties in obtaining a sterile product.

One of the many problems involved in the development of a suitable technic for radiation sterilization of foods is the selection of the type of radiation for processing. Both beta (fast electrons) and gamma radiation have been utilized. For beta radiation treatment a resonant transformer or Van de Graaff generator has been used. In gamma radiation treatment, radioisotopes such as cobalt^{60} and mixed fission products are the principal sources of this type of radiation. X-rays have also been used as a gamma ray source, but less efficiently, since a large amount of energy is dissipated in the form of heat. Beta rays having an

energy of 5 million volts are capable of penetrating the product one inch. Gamma rays of variant intensity have a much greater penetration distance into the product.

The dosage levels required for treatment or sterilization of a product are dependent upon the microbial population, the type of organism, chemical composition of the food and other factors. For the destruction of spoilage microorganisms it is common practice to utilize a range varying from 1,500,000 to 4,000,000 roentgen equivalents physical (rep). H. J. Gomberg, S. E. Gould, J. V. Nehemias, and L. E. Brownell (*Nucleonics* **12,** *No. 9, 66 (1954)*) have shown that with a maturation-inhibiting radiation dose of 15,000 rep the trichina larval cycle in pork could be broken. By the same token insect infestation of cereal products, a major problem with military rations, can be conveniently controlled by radiation treatment at 10,000 rep. Certain other aspects of radiation treatment of foods are also of interest. For example, the shelf life of prepackaged meats may be extended five-fold by low levels of radiation (about 50,000 rep). A. H. Sparrow and E. Christensen (*Ibid.* **12,** *No. 8, 16 (1954)*), utilizing a radiation dosage of 10,000 rep, have inhibited the sprouting of potatoes. These are all recent and important applications of radiation sterilization.

Many problems need to be solved before radiation sterilization of foods becomes commercially feasible. Objectionable off-flavors, odors, colors and undesirable textures are produced by definite chemical alterations in foods as the result of ionizing radiations. Consideration must also be given to costs of shielding and potential hazards from the radiation process and, last but not least, the large effect on nutritive quality of the processed food.

One major difference between the "cold sterilization" and heat sterilization of foods is that the latter procedure not only destroys spoilage microorganims but inactivates the enzymes capable of causing decomposition on subsequent storage. In radiation sterilization of food a radiation dosage exceeding 5 million rep must be utilized for enzyme inactivation. Failure to inactivate the enzymes may bring about a breakdown in fats, proteins and other constituents in the food during storage, characterizing the product as unacceptable due to enzymatic degradation.

Rather extensive investigations have been undertaken on the changes in nutritive value of foods exposed to high energy radiations. H.-C. Kung, E. L. Gaden, and C. G. King (*J. Agr. Food Chem.* **1,** *142 (1953)*; *Nutrition Reviews* **12,** *138 (1954)*) in working with milk and milk products demonstrated that vitamin A, tocopherols, and ascorbic acid were highly sensitive to gamma radiation at the sterilization dose. At the same radiation level the carotenoids and riboflavin were moderately sensitive, but the enzyme phosphatase, sensitive to heat treatment, was only slightly changed. Considerable losses in the micronutrients in such products as cream cheese and cheddar cheese resulted when they were irradiated at 80,000 roentgens per hour before complete sterilization was accomplished. Ascorbic acid destruction by ionizing radiation has been studied extensively. B. E. Proctor and S. E. Goldblith (*Nucleonics* **5,** *No. 3, 56 (1949)*) have shown that 74 per cent of this essential nutrient, in aqueous solution at a concentration of 50 micrograms per milliliter, was destroyed when irradiated at 75,000 roentgens. In pure solution, ascorbic acid is more radiosensitive than niacin and riboflavin, while riboflavin is the least radiosensitive of the three. Furthermore, when niacin and ascorbic acid, together in pure solution, are irradiated with a 50 kilovolt x-ray source, the niacin exhibits some sparing action on the ascorbic acid. It is believed that in this case the niacin takes on more readily than ascorbic acid the molecules of activated water. Vitamin B_{12} in pure solution has been shown to be even more radiosensitive than ascorbic acid (Proctor and Goldblith, *loc. cit.*).

Whereas such changes as described above

may occur in pure solutions, the decomposition of labile vitamins in a complex system such as a food may not be comparable, due to the presence of naturally occurring protective substances or radioresistant compounds. It has been noted that sulfhydryl compounds are extremely sensitive to ionizing radiation; hence cysteine, like ascorbic acid which is radiosensitive, can be used as a free radical acceptor in accomplishing the elimination of off-flavors in foods. By the same token, methionine has a protective effect on niacin in aqueous solution when treated with cathode rays. High concentrations of essential unsaturated fatty acids have an accelerative effect on the autoxidation of essential metabolites.

During irradiation some degradative changes occur in the macronutrients, especially fats, which are susceptible to oxidation and may yield peroxides, while proteins with sulfur-containing dipeptides may rupture and yield hydrogen sulfide and mercaptans. In meats such compounds have been produced by irradiation along with pigmental changes which not only give rise to discoloration but result in off-flavors and odors. Certain materials such as turmeric, mace, pepper, sage and onion can be added to foods to mask these undesirable organoleptic properties.

Despite the fact that considerable impetus has been given to developmental research in the technology and chemistry of ionizing radiations on foods, only recently have extensive animal feeding studies been initiated concerned with the nutritional and physiologic aspects of feeding irradiated foods to test animals. As early as 1948, studies made on the feeding of a capacitronized (treated in a capacitron) ration to rats showed that there was an impairment in the fertility of the male and increased mortality in litters which probably was due to destruction of vitamin E (E. DaCosta and S. M. Levenson, *Med. Nutrition Lab. Report No. 89, Dec. 14, 1951*). These findings were confirmed in other laboratories.

In the military a broad contractual research program is currently supported by the Department of Defense. Collaborating in this program are the Office of the Quartermaster General and the Office of the Surgeon General, Department of the Army. Whereas the former technical service has been concentrating on the technologic problems involved in the development of suitable irradiated foods, the Office of the Surgeon General supports research relating to the nutritional and probable toxicologic aspects of feeding irradiated foods. In brief, consideration is given to acute and chronic feeding experiments, studies on reproduction and lactation, carcinogenicity, effects on macronutrients and micronutrients, and general toxicity clearance of such foods on rats, dogs, chickens, monkeys, and finally, human test subjects. The health of the ultimate consumer of irradiated foods must be safeguarded by such wholesomeness studies as delineated above.

Great strides are being made in perfecting the "cold sterilization" method of food processing. While the radiation sterilization process in itself may yield an undesirable product, it is highly possible that a mild preheat treatment (inactivation of enzymes) prior to irradiation may be the accepted method of processing within the next five years.

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ANEMIA IN ESKIMOS

A considerable portion of the Alaskan Eskimo population is moderately anemic by usual standards. A recent paper of E. M. Scott, R. C. Wright, and B. T. Hanan (*J. Nutrition* **55,** *137 (1955)*) presents work directed toward the characterization of the

anemia and a determination of its origin. Fingertip blood was used for analyses which included the measurement of hemoglobin, serum iron, serum copper, hematocrit, red cell counts, and cell diameter.

The hemoglobin values of 715 Eskimo men between the ages of 17 and 50 who were members of the National Guard from numerous villages along the western coast of Alaska were obtained. For analysis the data were divided into Northern (297 men) and Southern (418 men) Eskimos and compared with data on 110 white soldiers stationed in Anchorage. The mean value for the latter group was 15.76 g. of hemoglobin per 100 ml. of blood and the mean values for the Northern and Southern Eskimos were 14.91 and 14.60 respectively. Histograms of the Eskimo data show a general shift toward lower hemoglobin levels. The lowest value obtained in the 715 men was 10.9 g. per cent.

Four villages in southwestern Alaska were selected for further study. Two of these had shown rather low hemoglobin levels for men in a previous survey and in the other two high mean levels had been found. The adult males and females tended to have low hemoglobin values in three of the towns. Somewhat higher values were observed in the fourth where the National Guard personnel previously had high hemoglobin levels. In the three towns where anemia was most prevalent selected groups of males and females were studied intensively. From these groups a few men with values above 14 g. per cent and women with values above 12 g. per cent were excluded, but few were above these levels. Complete blood indices were obtained. In men the anemia was moderate with only a slight tendency toward microcytosis. The same was found in many women, but 15 per cent of the women had hemoglobin levels below 10, and 6 per cent were below 8 g. per cent. Average values for women between the ages of 20 and 40 were considerably lower than those for older or younger women. In women with the lowest hemoglobin levels the anemia was definitely microcytic and hypochromic. Serum iron values were quite low in most of the groups studied, between 25 and 50 micrograms per cent compared to values of approximately 100 in white males and females in Anchorage. Average serum copper values in different areas, 114 to 157 micrograms per cent, were somewhat higher than those of the average adult Anchorage whites (102 to 104 micrograms per cent).

A therapeutic experiment was tried in the selected groups (males with less than 14 g. per cent hemoglobin and females with less than 12 g. per cent hemoglobin). The subjects were screened by x-ray to eliminate those with tuberculosis, and pregnant women were excluded. The therapy consisted of either 0.65 g. of ferrous sulfate, 0.66 g. of calcium lactate, or 100 mg. of ascorbic acid. The pills were distributed in limited amounts with instructions to take one daily and to return when they were gone for more. If the individuals returned, it was assumed that the pills had been consumed. From 9 to 14 individuals in each group were apparently considered to have followed the therapy. During the six-week trial the only significant effect observed was a rise in serum iron in the ferrous sulfate group. One woman of this group who originally had a hemoglobin level of 5.6 showed an increase to 10.8 g. per cent.

Dietary histories were not obtained, but in three of the villages the diet was composed largely of wheat flour, seal oil, fish and sugar. The kind of fish varied in the three villages, but was usually eaten uncooked. Meat was generally unobtainable during January to March, when the studies were done, although variable amounts and kinds (mink, muskrat, moose, seal) were available in the fall or spring. The fourth village was quite different from the others. The families were larger, housing was better, gardens were kept by some families, and some families canned meat, fish and vegetables for winter use. Local potatoes were a common food. This

does not necessarily mean that the diet was better, and if it was, it was not reflected in higher hemoglobin levels.

The low hemoglobin levels did not appear to be due to diphyllobothriasis. Although the prevalence of this parasite varied from 4 to 13 per cent in the villages studied, the presence of the tapeworm was not associated with hemoglobin levels lower than average and those persons infected did not show microcytosis or eosinophilia. The authors suggest that the anemia is of nutritional origin on the basis of the following observations: (1) the diets were severely restricted; (2) the school children all received a hot breakfast of soup, beans, rice, or oatmeal plus some additional food, and this group had more nearly normal hemoglobin levels than the adults; and (3) the National Guard personnel who had high hemoglobin levels in one village in contrast to the rest of the village adults had but recently returned from the Pribilof Islands where they had a high quality diet typical of that eaten in the United States. That iron deficiency may have been responsible is indicated by the nature of the anemia in many cases and by the low serum iron levels. On the other hand, the diet composed largely of fish and enriched flour should have had a fair iron content, and it is difficult to conceive of the intake being lower than 10 mg. on this diet. Also, in one village where the anemia in women was as severe as in the others, there was much less microcytosis. The results of the iron therapy experiment, although limited, do not indicate a marked iron deficiency. Genetic factors are not considered as a likely explanation in view of the hemoglobin levels obtained in some of the National Guard men, particularly those who had previously received a good diet. In some of the villages the people were closely related while in others they had widely scattered origins.

The authors conclude that although iron deficiency exists and perhaps largely explains the microcytic hypochromic anemia in women, there is a considerable portion of the population with a moderate normochromic, normocytic anemia and that some other factor, probably nutritional, is the cause. Careful dietary studies as well as more extensive and better controlled therapeutic tests should be informative. The so-called "natural" experiments offer opportunities that cannot be duplicated elsewhere.

VITAMIN B_{12} IN JAMAICAN CHILDREN

S. J. Patrick (*J. Nutrition* **55,** *129 (1955)*) has studied the serum level and the metabolism of vitamin B_{12} after administration of the vitamin. The studies were done upon Jamaican children of mixed African descent between 8 and 15 years of age who had a low intake of animal protein, and it was thought that they might be deficient in vitamin B_{12}.

Thirty-eight children from the same village were divided into two comparable groups with respect to age, sex and economic status. One group received a troche containing 100 micrograms of vitamin B_{12} five days a week over a nine-month period. The total consumption during the experimental period averaged 13.9 mg. per child. The control group received placebos. The serum concentrations of the vitamin were measured during the last month of the study using the method of H. L. Rosenthal and H. P. Sarett (*J. Biol. Chem.* **199,** *433 (1952)*). In the unsupplemented group the serum values ranged from 0.008 to 0.043 micrograms per cent, average 0.021. Comparable figures for the vitamin B_{12} group were 0.007 to 0.90, with an average of 0.033 micrograms per 100 ml. of serum. Although the vitamin B_{12} administration produced a significant rise in the serum level it was

small considering the rather massive dose used. It is of interest that the unsupplemented group had vitamin B_{12} levels in serum very similar to those found in healthy American adults by Rosenthal and Sarett (*loc. cit.*).

In an attempt to saturate the blood of 2 children, 1.5 micrograms of vitamin B_{12} per kilogram of body weight was injected intravenously on the first, fifth, eighth, twelfth, and fifteenth days of the experiment. Blood samples were drawn just before each injection and urine was collected during zero to five hours and five to seven hours after the injections. The serum level prior to the first injection was 0.014 micrograms per cent. This increased to 0.07 after one injection and to 0.13 per cent after the second. No further increase occurred, and the serum level fluctuated between 0.10 and 0.12 micrograms per cent. After each injection approximately 40 per cent of the dose was excreted in the urine in the following five hours and practically none (0.05 to 0.15 per cent) in the next two hours. Further studies showed that practically all of the vitamin excreted was excreted within the first two hours. The more rapid rate of excretion found in these studies than by other authors (D. C. Chesterman, W. F. J. Cuthbertson, and H. F. Pegler, *Biochem. J.* **48,** *li* (*1951*)) is probably due to the method of administration.

Plasma levels and urinary excretion at intervals after the injection of vitamin B_{12} in two groups of children, one judged normal and the other failing in growth as judged by the Wetzel grid, were also studied. The test dose was 1.5 micrograms per kilogram of body weight. No statistically significant differences in the original level of vitamin B_{12}, the serum levels at intervals after injection, or the excretion rate were observed. The serum level was considerably higher for as long as six hours after the injection (0.08 to 0.09 micrograms per cent compared to the original level of 0.03), although the urinary excretion dropped promptly to very low levels, as indicated above.

The complexities of the metabolism of vitamin B_{12} have been commented upon previously (*Nutrition Reviews* **12,** *105* (*1954*); C. A. Lang, D. M. Gleysteen, and B. F. Chow, *J. Nutrition* **50,** *213* (*1953*)). It appears that neither serum levels nor response to load tests, as measured by change in serum level or urinary excretion, are indicative of the vitamin B_{12} intake, except when intakes are very high. Whether the children treated with the vitamin were benefited in any way was not considered in this report.

CYSTINURIA, A RENAL TUBULAR DEFECT

Cystinuria is a disease of man characterized by a high urinary excretion of cystine, often with the formation of cystine stones in the kidneys and ureters. It was long described as an "inborn error of metabolism" and some thought that an increased production of cystine in the body was concerned. Spotty evidence has indicated that this may not be the case but rather that the disease might be renal in origin. This thesis has been well confirmed by C. E. Dent, J. G. Heathcote, and G. E. Joron (*J. Clin. Invest.* **33,** *1210* (*1954*)) and by Dent, B. Senior, and J. M. Walshe (*Ibid.* **33,** *1216* (*1954*)). In the first study 2 normal individuals and 2 patients with cystinuria were investigated and in the second, 9 normal subjects were compared with 7 cystinurics.

In the first study 5 g. of L-cystine, 6.2 g. of DL-methionine and 6.5 g. of L-cysteine hydrochloride were fed, each on a separate occasion, and the plasma level and urinary output of cystine for the hour prior and the

seven hours following amino acid ingestion were determined by both chromatographic and microbiologic assay. Both the normal individuals and those with cystinuria showed a slight rise in plasma cystine levels following the ingestion of the cystine and methionine. No cystine excretion occurred in the normal subjects, but there was the expected continued excretion of cystine by the cystinuric patients. Following cysteine ingestion, there was a sharp rise in plasma cystine level during the first hour, following which it dropped off to or toward normal. Cystine appeared for the first time in the urine in the normal individuals and increased greatly in amount in the cystinuric subjects following cysteine ingestion. The plasma levels of cystine seemed somewhat higher in the patients with cystinuria than in the normal individuals after cystine administration. The urinary output of cystine appeared to follow the cystine blood levels, both in the cystinuric subjects and in the normal individuals when cysteine had been given.

The authors believed that the sharp rise in blood cystine content after cysteine but not after cystine administration was due to the relatively greater solubility of the former over the latter and hence a much more rapid absorption and conversion of cysteine to cystine. The relatively close relation between the urinary cystine and plasma cystine levels and the degree of cystine excretion in the urine, depending upon whether the individual was a cystinuric subject or not, led the authors to conclude that the cystinuria is better explained on the basis of a defect in kidney tubule function than as an inborn error of metabolism. This is further supported by the finding in patients with cystinuria of large quantities of lysine, arginine and perhaps other amino acids as well as cystine in the urine (C. E. Dent and G. A. Rose, *Quart. J. Med.* **20,** *205 (1951)*).

In order to develop a more quantitative method for cystine, and particularly to calculate renal clearances, cystine was determined by polarography. The authors discuss thoroughly the limitations of this method and believe that, although it may not give absolutely reliable figures in every case, any errors would be systematic and would not affect the conclusions reached. Changes in blood and urine cystine after feeding cystine, methionine, and cysteine to normal and cystinuric subjects were essentially the same using this method as those found in the first study. Of great importance, however, was the finding that the endogenous clearance of cystine was about thirty times higher in the cystinuric than in the normal individuals, even though the former subjects had blood levels slightly lower than the latter individuals.

When DL-cystine was fed there was a marked increase in cystine clearance in normal individuals, but not in the cystinuric patients. It was believed by the authors that this was because the normal tubule rejected the natural isomer of cystine whereas in the cystinuric subject both natural and unnatural isomers were not absorbed and appeared in the urine.

The authors concluded, with good justification from the data presented, that the cystinuria in this pathologic condition resulted from "greatly diminished (or absent) cystine reabsorption by the renal tubule."

MILK-ALKALI DISEASE

It is usual in the treatment of peptic ulcer to provide a diet affording bland foods and milk, and antacids to reduce pain and gastric acidity. That such a program of treatment could lead to difficulty if not properly controlled was indicated in a

report in 1949 by C. H. Burnett, R. R. Commons, F. Albright, and J. E. Howard (*New Engl. J. Med.* **240,** *787 (1949)*). The occurrence of increased concentration of serum calcium, deposits of calcium in the membranes of the eye and in other tissues, and kidney damage were described in patients who had ingested large quantities of milk and readily absorbed alkali. These events were not associated with increased urinary output of calcium or with alkalosis.

Since the early description of this disorder similar cases have been recognized by other observers. Two recent reports deal with the syndrome and its recognition. One of these is by D. A. Scholz and F. R. Keating (*Arch. Int. Med.* **95,** *460 (1955)*) and describes the disorder in 8 patients observed at the Mayo Clinic. The medical histories of all of the patients indicated that they had had symptoms of peptic ulcer persistent or recurrent over periods of many months to several decades. All but one consumed in excess of a quart of milk a day. Several ingested as much as 3 to 4 quarts daily. Each of the patients also took frequent doses of alkaline powders. Many of these preparations included calcium carbonate. Astounding quantities of antacids were consumed by several patients. For instance, one man estimated his weekly intake of Sippy powders (calcium carbonate and magnesium oxide) at 2 to 2½ pounds. It was not unusual for the patients to state that for weeks or months they had virtually lived on milk and alkaline powders. The complaints of the patients when first seen gave little or no indication of the associated disorder. Findings on physical examination were few and in no way designated the type of underlying metabolic disturbance. Unlike other patients with the milk-alkali syndrome described in the medical literature, deposits of calcium in the conjunctiva and cornea of the eyes were not noted. It was only by laboratory procedures that the diagnosis was achieved. In all of the patients there was increase in serum calcium. Values ranged from 11.8 to 15.6 mg. per cent. The normal range for serum calcium is from 9.0 to 11.0 mg. per cent. In contrast, concentrations of serum inorganic phosphorus were increased in but 2 of the patients. An elevation in the urea content of serum in each patient afforded evidence of nitrogen retention and kidney dysfunction. Only in those who had been vomiting and were dehydrated were there blood chemical changes of alkalosis. Perhaps the most remarkable characteristic of this disorder is the relatively rapid correction of the associated metabolic defects with modification of diet and treatment. The procedure utilized was to prescribe an ulcer diet, eliminating milk and with low calcium content, and to give nonabsorbable alkalis, such as aluminum hydroxide gel, in place of the more readily absorbed alkaline powders. Under this regimen serum calcium levels were reduced to normal in from four to ten days in most of the patients. Simultaneously, there was decrease in blood urea and improvement in kidney function.

A second paper describes 3 additional cases of the milk-alkali syndrome and reviews the literature on the subject (E. Kessler, *Ann. Int. Med.* **42,** *324 (1955)*). The cases are comparable with those described by Scholz and Keating (*loc. cit.*). Most of the subjects with the syndrome described in the literature have exhibited localized deposits of calcium in tissues. Most characteristic have been crescentic lesions at the margins of the cornea and small crystalline calcium masses in the conjunctiva. However, other sites have been involved as well. Included are calcium deposition in kidney tubules, in subcutaneous tissues, in the lungs and in arterial walls.

In the diagnosis of the milk-alkali disorder certain other processes producing hypercalcemia and localized deposits of calcium must be considered. Hypervitaminosis D is such a disorder (*Nutrition Reviews* **3,** *313 (1945)*). The excessive intake of vitamin D leads to increased absorption of calcium and phos-

phorus from the intestinal tract, elevation in serum calcium content, increased urinary excretion of calcium, calcium deposition in various tissues, and kidney damage. Clinical characteristics may make it very difficult to differentiate between hypervitaminosis D and the milk-alkali syndrome. However, a history of long-continued and massive intake of the vitamin makes consideration of the hypervitaminotic state imperative. Moreover, limitation of calcium in the diet and exclusion of vitamin D will ordinarily bring about prompt correction of the hypercalcemia of the milk-alkali syndrome, while this change occurs much more slowly in hypervitaminosis D.

Hyperfunction of the parathyroid glands may also produce hypercalcemia and the other metabolic defects associated with the milk-alkali syndrome. However, there is usually a marked loss of calcium in the urine of patients with this disorder, a finding which should be helpful as a diagnostic consideration. Still other diseases may induce similar manifestations. Included are metastatic carcinoma with involvement of bone, multiple myeloma, sarcoidosis, and acute osteoporosis.

The mechanisms producing the disturbances which characterize the milk-alkali syndrome are not fully understood. If calcium salts in solution are injected intravenously or if calcium is mobilized from storage sites in bone, there is usually an outpouring of the element in urine. Why this does not occur in patients who develop the milk-alkali disorder is not readily explained. A number of factors may contribute, however. It is known that alkali administered by mouth will decrease the urinary rate of excretion of calcium. Similarly, the ingestion of inorganic phosphate reduces the output of calcium in urine. Since these patients have consistently consumed alkaline powders in relatively large quantities and have had large intakes of milk with its high content of phosphorus as well as calcium, it is reasonable to assume that both factors are important. Still another mechanism may be involved, however. This is kidney damage. Most, if not all, of the patients described have had evidences of renal dysfunction. In some, at least, the medical histories are indicative of pre-existing kidney disease. The damaged kidney may have a reduced capacity to excrete calcium and may thereby permit the accumulation of the element in blood. Unfortunately, the evidence at hand does not permit the deduction that previously established renal disease is necessary for the induction of the milk-alkali syndrome. Actually, there is improvement of kidney function in most of the patients after calcium and absorbable alkali are removed from the diet or restricted. A number have recovered without any type of residual kidney damage, while others have continued to manifest evidences of renal disease. It remains difficult, therefore, to assess fully the role of kidney damage in the evolution of this disorder.

The milk-alkali syndrome is one of interest and results from the efforts of the physician and patient to achieve relief from the distressing pain and other symptoms of a very common disease. It would be unfortunate, indeed, to impugn a valuable and good food, milk, because of the occasional occurrence of this disorder. Its place in the diet of the patient with peptic ulcer should be retained. It would seem to be of greatest importance, however, to restrict the use of alkaline powders, since these are readily absorbable. It may be unfortunate that these are so easily obtained as baking soda or proprietary ulcer remedies. On the other hand, it is fortunate that antacid preparations are available which afford almost as prompt and complete relief from the distress of peptic ulcer without the hazard of inducing the milk-alkali disorder. These are the nonabsorbable alkalis, examples of which are aluminum hydroxide and magnesium trisilicate.

METABOLISM FOLLOWING BURNS

Disturbances in the metabolism of all nutrients so far studied have been observed following severe burns (*Nutrition Reviews* **9,** *257* (*1951*)). Hyperglycemia, glycosuria, lactacidemia, and lowered plasma carbon dioxide-combining power usually appear soon after injury and persist for a number of days. In general, the extent of these changes parallels the severity of the burn.

It appears that there is a marked increase in carbohydrate turnover. Decreases in muscle and liver glycogen following burns have been described in animals. Similar measurements have not been made in patients. An increase in lactic acid production due to an increase in the rate of muscle glycogenolysis may result in hyperglycemia through conversion of the lactic acid to glycogen and glucose in the liver. The rate of lactic acid formation following burns may be greater than the liver can handle, resulting in lactacidemia as well as hyperglycemia. In addition to increased glycogenolysis, another possible source of the extra blood sugar is an increase in gluconeogenesis. Following thermal burns, there is an early increase in protein breakdown, with the excretion of excessive amounts of nitrogen in the urine. Some of this protein may be converted to glucose.

Hyperglycemia and lactacidemia are not peculiar to burn injury but have been reported following other types of trauma, hemorrhagic shock, acidosis, anhydremia (deficient fluid in the blood), and low oxygen tension. J. D. Robertson (*J. Physiol.* **84,** *393* (*1935*)) has presented evidence that in cats the liver is the site of formation of the extra glucose after hemorrhage; the sugar content of the hepatic vein blood was higher than that of the heart blood, and ligation of the hepatic vessels prior to bleeding prevented the hyperglycemia. C. M. Brooks (*Am. J. Physiol.* **114,** *30* (*1935*)), working with chronic "spinal" cats, showed that an intact sympathetic nervous system is necessary for the production of the hyperglycemia following hemorrhage.

The possible role of the adrenal glands in the production of abnormalities in carbohydrate metabolism after burns has been emphasized. An early transient increase in epinephrine production and a more sustained increase in adrenocortical activity have been observed. In burned rabbits, E. J. Clark and R. J. Rossiter (*Quart. J. Exp. Physiol.* **32,** *279* (*1944*)) found that neither the lactacidemia nor the hyperglycemia was completely abolished. Hyperglycemia has been observed following traumatic shock (H. Selye and C. Dosne, *Proc. Soc. Exp. Biol. Med.* **48,** *532* (*1941*)) or diethylstilbestrol administration (D. J. Ingle, *J. Clin. Endocrinol. Metab.* **14,** *1272* (*1954*)) in adrenalectomized rats receiving a constant maintenance level of adrenocortical hormones. T. W. Burns and F. L. Engel (*Am. J. Physiol.* **167,** *772* (*1951*)) have found that administration of cortisone is associated with a greater impairment of glucose tolerance in ill than in healthy subjects. It would appear that the pituitary and adrenals play an important role in the production of abnormalities in carbohydrate metabolism following burns, but it is likely that other factors are also involved. In patients extensively and deeply burned changes in liver function are seen. No conclusive functional or anatomic changes have been reported as occurring in the pancreas or thyroid (*Nutrition Reviews* **11,** *265* (*1953*); **12,** *265* (*1954*)).

W. J. H. Butterfield (*Lancet* **I,** *489* (*1955*)), in a preliminary communication, has reported hyperglycemia, glycosuria (but not acetonuria), impaired glucose tolerance and insulin resistance in some patients with severe burns who were force-fed (details of dietary intake are not given). The abnormal carbohydrate metabolism lasted days, weeks,

or months. Insulin resistance was most marked in the first three weeks after injury, but persisted, in some instances, up to thirteen weeks. Thereafter, if hyperglycemia was present, it was insulin-sensitive. Increase in insulin sensitivity in the early hours following burns has been reported by F. H. L. Taylor, S. M. Levenson, and M. A. Adams (*New Engl. J. Med.* **231,** *437 (1944)*) in 2 burned patients as judged by blood glucose levels, and by C. L. Henry, R. J. Lichter, and J. C. Daw (*Surg. Gynec. Obstet.* **100,** *265 (1955)*) in dogs as judged by serum potassium levels.

Butterfield (*loc. cit.*) has noted that the concentrations of blood keto acids were elevated in burned patients with hyperglycemia and rose to somewhat greater levels after glucose administration than in burned patients without hyperglycemia or in force-fed control patients. Smaller differences in the changes of the blood levels of the keto acids were observed after glucose-insulin administration, except for a greater rise in the 2 burned patients with hyperglycemia more than thirteen weeks after injury.

Butterfield found that 9 of the 15 burned patients with hyperglycemia showed increased excretion of urinary corticoids and depressed eosinophil counts over the first five days after injury. Since some of the metabolic effects of increased adrenocortical activity may be related to changes in sulfhydryl (SH) metabolism (*e.g.*, intravenous glutathione reduces hyperglycemia caused by corticotropin administration), Butterfield studied the effect of dimercaprol (BAL) in the burned patients, some of whom were receiving corticotropin, and in his control patients. Dimercaprol, 250 mg. in oil, was given intramuscularly at the start of the glucose tolerance tests (50 g. of glucose, administered orally) or the glucose-insulin tolerance tests (50 g. of glucose given orally, 5 units of insulin administered intravenously). The glucose-dimercaprol tolerance tests were carried out during the first three weeks after injury and in the period three weeks to thirteen weeks after burns. These tests were carried out only in the period three to thirteen weeks after injury. The blood keto acids and sugar concentrations rose less following the glucose-dimercaprol administration than following the ingestion of glucose alone. Dimercaprol administration had less apparent effect in the glucose-insulin tests.

The effect of more prolonged administration of dimercaprol was studied in 7 diabetic subjects, whose individual insulin requirements varied from 24 to more than 320 units daily. They were kept on regulated diets and insulin. After control periods of two to five days, they were given intramuscular dimercaprol, 5 per cent in oil, 4 ml. four times daily for three days. They were then followed for a number of days after discontinuance of dimercaprol. In 5 of the 7 patients (including the two with the highest insulin requirments), hyperglycemia and glycosuria diminished during the period of dimercaprol administration, and then returned to previous levels. In one patient with a high daily insulin requirement (280 units), dimercaprol was given for twelve days at a daily dosage level of 4 ml. of a 5 per cent solution intramuscularly and then for eight days at a level of 8 ml. daily. His daily urinary glucose excretion was 11 g. during a thirty-seven-day control period, 8 g. during the first dimercaprol period (4 ml. daily), and 2 g. during the second dimercaprol period (8 ml. daily).

In two studies made so far, Butterfield has found no laboratory evidence of concomitant depression of adrenocortical activity during the period of dimercaprol administration. Butterfield indicates that his studies are continuing in an attempt to analyze the disturbances in glucose metabolism in burned and diabetic patients which appear to be corrected by dimercaprol.

NUTRITION AND HEALTH OF AN AGED POPULATION

Although there are a number of longitudinal nutritional studies of children and young adults, there are relatively few which encompass older people. Preliminary reports have appeared on a five-year study of 577 men and women over 50 years of age. The study was started in San Mateo County, California, in the summer of 1948 (H. D. Chope and S. Dray, *California Med.* **74,** *105 (1951)*). It was a cooperative project involving the Bureau of Human Nutrition and Home Economics of the U. S. Department of Agriculture, the U. S. Public Health Service, the Department of Home Economics of the College of Agriculture of the University of California, the California State Department of Public Health, and the Department of Public Health and Welfare of San Mateo County.

Individuals selected for the study were residents of San Mateo County (located just south of San Francisco), who were physically and mentally well, were not on any special diet, and who had not been under the care of a physician for the previous three months. There were 280 men and 297 women. The ages ranged from 50 to 89, with an approximately equal distribution in all but the last decade (only 5.7 per cent of the group were 80 to 89). Although there were a few subjects in the lower and higher economic groups, by far the largest percentage were in the middle economic group.

The examinations on each subject included a nutritional history covering both a seven-day dietary record and a general food history; a medical history and medical examination with special emphasis on nutritional status; laboratory tests of blood samples for hemoglobin, serum protein, ascorbic acid, vitamin A, carotene, and cholesterol; and x-rays, including bone density determination according to the method of P. B. Mack (*see Nutrition Reviews* **10,** *119 (1952)*).

Hypertension, as indicated by systolic blood pressures above 200 mm. of mercury, was the most common difficulty seen. It occurred in 20.8 per cent of the people. Obesity was the next most common medical abnormality; 17.5 per cent of the subjects were 16 per cent or more above the calculated average weight for age and sex. The incidence of overweight was only slightly higher among the females than among the males. There was a definite correlation between obesity and hypertension. Among the overweight subjects 23.8 per cent were hypertensive, while only 11.6 per cent of those who were not overweight were in that group.

The incidence of anemia in this group was surprisingly small. Only 2.4 per cent of the subjects had hemoglobin levels of 11 g. per cent or less. The average hemoglobin level for the men up to 75 years of age was 14.5 g. per cent, while that for the women was 13.4 g. per cent (A. F. Morgan and H. L. Gillum, *Fed. Proc.* **13,** *469 (1954)*). According to Morgan and Gillum, these means are 1 to 2 g. per cent lower than those in young adults. Above age 75 the levels for males and females decreased and were similar. There was a slight positive correlation between hemoglobin levels and dietary iron (0.12) and between hemoglobin level and dietary protein (0.13).

In the summer and fall of 1952, a survey of the same group was carried out (H. D. Chope, *California Med.* **81,** *335 (1954)*). During the four-year interim, 49 of the original subjects died. Half of the people died from heart and circulatory diseases, while nervous system diseases and neoplasms accounted for most of the others. A study of the findings in the original examination showed that there was no relationship between the death rate and the following factors: hemoglobin, blood glucose, blood creatinine, and intake of calories, protein, fat, calcium, iron, or cholesterol.

There was a higher than expected incidence of mortality among the individuals who had blood cholesterol levels below 220 mg. per cent than among those with levels above that figure. Approximately 15 per cent of the people who had low cholesterol levels at the time of the first examination died, while only 11 per cent with the higher cholesterol levels died. The causes of death in these cases are not given.

There were suggestions of a lower mortality among the individuals who on the first examination reported high intakes of vitamin A, niacin and ascorbic acid.

Among the 306 subjects who completed the second examination, only 78 (25 per cent) reported they had not been ill during the four-year interval. The illnesses reported were almost equally distributed among those involving the nervous system, circulatory system, respiratory system, digestive system, and muscle-skeleton. There was a high incidence of respiratory diseases among the subjects with low hemoglobin levels (less than 13 g. per cent). The incidence of digestive disturbances was high among the persons with hemoglobin levels above 15 g. per cent.

It is rather surprising that these workers reported a lower incidence of circulatory diseases among the individuals with a higher caloric intake. Unfortunately, the report gives no data to support the statements made. For this reason it cannot be determined whether the individuals with the higher caloric intake were obese.

The report states that the protein intake had little effect on the incidence of illnesses of the nervous or circulatory systems. However, among subjects whose protein intake was between 60 and 79 g. a day, the incidence of respiratory diseases was high.

Subjects with a vitamin A intake below 5000 I.U. per day had a high incidence of nervous, circulatory and respiratory diseases, while the incidence of diseases of the digestive and musculoskeletal systems was low. Individuals with an intake above 8000 I.U. per day had a low rate of nervous and circulatory diseases.

Thiamine intakes less that 0.8 mg. per day appeared to be associated with a higher incidence of nervous and circulatory diseases.

Among the subjects examined at the time of the follow-up, there was a high incidence of nonfatal circulatory diseases in the people who had high levels of cholesterol in the blood. However, the individuals with a high cholesterol intake (760 mg. a day or more) had fewer circulatory diseases than the average for the entire group.

It can only be hoped that papers soon will be forthcoming providing the data upon which the above statements are based. A study such as this, with its far-reaching implications, should be subjected to as much critical scrutiny as possible. If some of the above conclusions are correct, this study will provide those interested in nutrition education with startling documentary evidence. The study is important because it attempts to correlate health over a period of time with nutrient intake data. As such it is one of a number of pioneer studies.

IMPROVED PROCEDURE FOR ESTIMATING FORAGE DIGESTIBILITY

In conventional methods for determining the digestibility of feeds, total feed intake and total fecal output of the various feed constituents (crude protein, crude fiber, ether extract, ash, and nitrogen-free extract or carbohydrate) must be measured over a period of seven to ten days. Research in animal nutrition in recent years has been directed toward methods that would circumvent the need for total feed intake and total fecal output information. Various investigators have used ratio technics to establish the digestibility of a given feed. For example, the ratio of feed lignin to fecal lignin permits

calculation of the extent of removal of digestible constituents of a feed during passage through the digestive tract. If the feed intake is known and an inert substance such as chromium oxide is introduced with the feed, it is also possible to calculate digestibility of feed constituents from the ratio of chromium oxide in feed to chromium oxide in feces (E. A. Kane, R. E. Ely, W. C. Jacobson, and L. A. Moore, *J. Dairy Sci.* **36,** *325 (1953)*).

Several investigators have also suggested that feed consumption of grazing animals could be determined by taking advantage of a constant relationship between fecal nitrogen excretion and dry matter intake (K. L. Blaxter and H. H. Mitchell, *J. Animal Sci.* **7,** *351 (1948)*; W. D. Gallup and H. M. Briggs, *Ibid.* **7,** *110 (1948)*; R. J. Lancaster, *New Zealand J. Sci. Technol.* **31,** *1, 31 (1949)*). Such a procedure, combined with the introduction into the digestive tract of known amounts of an inert material such as chromium oxide, would permit the estimation of dry matter digestibility of forages in grazing animals by sampling and analyzing feces alone. R. M. Forbes (*J. Animal Sci.* **8,** *19 (1949)*), however, claimed that total fecal nitrogen varied too widely to be of practical value as an estimate of feed intake by grazing animals.

An alternate procedure is one involving the use of an inert indicator (chromium oxide) and the natural pigments in forages as a means of measuring digestibility and consumption of forages by grazing animals (*Nutrition Reviews* **11,** *78 (1953)*). A mathematical relationship was established between the chromogen:dry matter ratio in feces and that of forages. This relationship circumvented the manual sampling of forages since the chromogen concentration of forages could be predicted from the fecal chromogen level. It was recognized that the chromogens involved were partially digested. However, since the chromogen concentration in feces was observed to be correlated with the digestibility of the forage consumed, it was assumed that this would not introduce a serious objection to the use of the procedure.

The chromogens or plant pigments that are measured in such a procedure are those that are extracted from the forage or fecal matter with a mixture of 85 per cent acetone (by volume) and 15 per cent water, and which are measured in terms of light absorption at the wave length 406 millimicrons. The chromogens are not a single entity but a mixture of pigments composed predominantly of chlorophylls and pheophytins, with small amounts of carotenoids (H. M. Irvin, H. G. Wiseman, J. C. Shaw, and L. A. Moore, *J. Animal Sci.* **12,** *541 (1953)*; W. W. G. Smart, F. W. Sherwood, G. Matrone, and G. H. Wise, *J. Agr. Food Chem.* **1,** *318 (1953)*).

Since the procedure lacked specificity in terms of compounds actually measured, workers at the North Carolina Agricultural Experiment Station have recently introduced a modification of the procedure to improve its specificity and useful range (W. W. G. Smart, G. Matrone, and V. W. Smart, *J. Agr. Food. Chem.* **2,** *1331 (1954)*). Earlier work had established that forages low in chloroplast pigments did not lend themselves to the use of the procedure (W. C. Cook and L. C. Harris, *J. Animal Sci.* **10,** *565 (1951)*). Smart and co-workers employ a preliminary treatment of the feed or fecal samples with acidified cupric chloride, which has the advantage of destroying the carotenoids and at the same time converting the chlorophylls into stable copper derivatives. Supplementary studies were conducted to ascertain some of the characteristics of the copper chlorophyll derivatives. It was found that they were stable in both acid and alkali. They were also stable to light and remained stable at room temperature for periods up to one week.

Transfer of the copper chlorophyll pig-

ments to ether, followed by chromatographic separation using a 1:1 magnesia-Supercel mixture, distinguished at least two components, one removable with ether and the other with pyridine. Their absorption maximums were at 422 and 650, and 410 and 650 millimicrons, respectively. Digestibility trials with rabbits established that the new procedure was particularly useful with forage materials low in chlorophylls and high in carotenoids. Measurement of only the stabilized copper chlorophyll derivatives eliminated the error introduced by direct chromogen measurement in acetone extracts containing considerable amounts of carotenoids that are metabolized or destroyed in passage through the digestive tract. The results would indicate that the chlorophylls are much more stable in the alimentary tract than are the carotenoid pigments.

The procedure recommended by Smart and associates appears to represent a real improvement over previous procedures. It further suggests that the ratio of stabilized chlorophylls in feed and feces should provide more accurate predictions of feed digestibility for a wider variety of forages than has previously been possible.

MOBILIZATION OF DEPOT FAT

The mechanism whereby the body draws upon its caloric reserves during periods of dietary inadequacy has always been intriguing and elusive. The means whereby the fat depots are activated to release or accumulate fat very likely involves either a nervous or a humoral impulse, or a combination of these. Much of the work on the nervous control of the adipose depots was done in the early part of this century. Most of the studies on the humoral control have centered around the endocrine secretions.

On the basis of clinical experience, a number of investigators concluded that abnormal nerve stimulation of one part of the body results in a loss of fat therefrom, while paralysis of a nerve was frequently followed by the deposition of excessive amounts of fat (quoted by E. Wertheimer and B. Shapiro, *Physiol. Rev.* **28,** *451 (1948)*). Previous to that, G. Mansfeld and F. Müller (*Pflügers Arch. f. d. ges. Physiol.* **152,** *61 (1913)*) showed that cutting the femoral nerve of the rabbit resulted in an increase in the fat content of that leg when compared with the intact leg.

More specific evidence for the role of the nervous system in regulating the movement of fat came from F. X. Hausberger (*Z. f. d. ges. exp. Med.* **102,** *169 (1937)*). He found that when the nerve to one of the interscapular fat depots was severed, there was an influx of fat and glycogen within ten hours. The fat content of the adipose depot on the denervated side exceeded that of the other side in both fed and fasted mice. Furthermore, it required a long time to deplete the denervated tissue of its fat content.

Adipose tissue is supplied with sympathetic nerve fibers which are reported to be different from those accompanying the blood vessels (quoted by I. G. Wool, M. S. Goldstein, E. R. Ramey, and R. Levine, *Am. J. Physiol.* **178,** *427 (1954)*).

The neurogenic control of fat migration to and from the depots has been summarized by H. J. Deuel ("The Lipids," *Vol. II, p. 618, Interscience Publishers, New York (1955)*) in the statement that: "It is believed that the status of an animal as regards obesity or leanness, as well as the localized deposition of fat is related to the extent to which it is subject to nervous stimuli."

While the above phase of the study was

being pursued less actively, another group of investigators approached the problem from the standpoint of the endocrine glands. A number of earlier investigators in this area reported that when large doses of posterior pituitary extracts were injected into rats or rabbits there resulted a deposition of fat in the liver (quoted by C. H. Best and J. Campbell, *J. Physiol.* **86,** *190 (1936)*). This work was not confirmed by Best and Campbell. In conjunction with their early studies on fatty livers, Best and Campbell (*loc. cit.*) prepared a protein-like extract from the anterior pituitary gland of beef cattle. Three days after this material was injected into normal female rats there was a reduction in carcass fat and an increase in liver fat. The source of the fat deposited in the liver was shown to be depot fat (H. M. Barrett, Best, and J. H. Ridout, *J. Physiol.* **93,** *367 (1938)*).

As a result of injecting the anterior pituitary extract into fasted mice, the reduction in body lipids was shown to involve primarily the glycerides (J. Campbell and C. C. Lucas, *Biochem. J.* **48,** *241 (1951)*). There was no change in the body cholesterol or cholesterol esters; there was a questionable reduction in the phospholipids. The lipids of the adipose tissue and the skin, in that order, were most heavily drained by the anterior pituitary extract. These depots were the ones that were reduced when mice were fasted. The increase in the liver lipids under these circumstances was associated with only a slight increase in the kidney lipids. These workers suggest that the rate of mobilization of lipid from a tissue depends upon the glyceride content of that tissue.

The name *adipokinin* was proposed for the anterior pituitary substance which accelerated the mobilization of depot fat (R. Weil and D. Stetten, *J. Biol. Chem.* **168,** *129 (1947)*). Weil and Stetten were unable to detect any changes in the concentration of adipokinin in the pituitaries of their rats following a period of starvation. They attributed their failure to the insensitivity of the assay method. However, when they concentrated the urine from rabbits that had been fasted for forty-eight hours, a preparation was secured which increased the liver fat level in mice. A similar preparation from ad libitum-fed rabbits had no such activity. No attempt was made to identify the urinary substance with that concentrated from the anterior pituitary by Best and Campbell (*loc. cit.*) other than the similarity in the concentration procedures.

Additional studies of the role of the pituitary in regulating fat mobilization were made by L. Levin and R. K. Farber (*see Nutrition Reviews* **10,** *351 (1952)*). They believe two pituitary hormones are involved, one being ACTH which stimulates the adrenal to produce increased amounts of 11-oxygenated steroids. The latter make the animal more responsive to the second pituitary hormone which they suggest as being probably the growth hormone (Levin and Farber, *Recent Progress in Hormone Res.* **7,** *399 (1951)*). They also state that fat mobilization in response to fasting does not occur in male mice in the absence of functioning testicular tissue.

Concurrently with the above reports there appeared a number of papers which implicated the adrenal gland in fat mobilization. Much of that work stemmed from the observation that the fatty liver resulting from phosphorus poisoning could be prevented by adrenalectomy (F. Verzár and L. Laszt, *Biochem. Z.* **288,** *356 (1936)*). Other workers showed that the fatty liver following a period of starvation in mice does not occur if the animals are adrenalectomized. The anterior pituitary extract of Best and Campbell does not increase the liver fat level in adrenalectomized rats or mice, and no fat accumulates in the hepatic remnant following partial hepatectomy in adrenalectomized rats (quoted by I. G.

Wool and M. S. Goldstein, *Am. J. Physiol.* **175,** *303 (1953)*).

Most of these studies emphasized the adrenal cortex. F. A. Hartman, K. A. Brownell, and J. S. Thatcher (*Endocrinology* **40,** *450 (1947)*) reported the isolation of a compound from the adrenal cortex which was distinct from the sodium and carbohydrate factors. This substance was reported to have caused the deposition of fat in the livers of adrenalectomized fasting animals. So far, this report has not been confirmed.

Recent work has brought the adrenal medulla into the picture. Wool and Goldstein (*loc. cit.*) used ethionine (an antimetabolite for methionine) for the production of fatty livers in female rats. The fatty livers did not occur when the animals were adrenalectomized. Cortisone, when used in small amounts, did not permit the mobilization of the depot fat as shown by liver fat levels. However, when the same levels of cortisone were given with epinephrine, liver fat increased as a result of ethionine treatment. Epinephrine alone was inactive.

In an extension of the above work, the influence of adrenalectomy and demedullation of the adrenals was investigated (Wool *et al., loc. cit.*). Adult female rats that had been adrenalectomized were maintained on cortisone and a stock diet. The animals were fasted for twelve hours before ethionine was injected. The fasting was continued for an additional twenty-four hours, at which time the rats were killed.

The injection of ethionine into the adrenalectomized rats produced a much lower liver fat level (7.4 per cent) than was seen in the ethionine-treated unoperated controls (14.6 per cent). The saline-treated controls had a liver fat level of 5.5 per cent. Although the injection of cortisone (in doses ranging from 0.4 to 10 mg. per rat) at the same time that ethionine was given raised the liver fat level (to a maximum of 9.9 per cent for the 4 mg. dose), the average never reached that of the unoperated controls.

Since the liver fat levels in some of the adrenalectomized rats treated with ethionine and cortisone did fall within the range for the unoperated rats, the study was extended to see if epinephrine might be involved in the reaction. To this end demedullation of the adrenal gland and ergotamine were used. (Ergotamine blocks the action of epinephrine.) When ergotamine was given to unoperated adult female rats together with ethionine, the liver fat level was much lower than in the rats treated only with ethionine (8.3 versus 14.6 per cent). In the adrenalectomized rats treated with cortisone, ergotamine together with ethionine resulted in liver fat levels that were equal to those in the unoperated rats given saline in place of ethionine (5.1 versus 5.5 per cent fat). When epinephrine was given to the demedullated rats receiving ethionine, the liver fat approached that of the unoperated controls given ethionine (12.5 versus 14.6 per cent).

On the basis of the above, Wool *et al.* "assign to epinephrine the role of effector agent in the mobilization of fat and to cortisone that of providing a facilitating background for this process."

These studies on the mobilization of depot fat are of importance in providing an insight into the possible origin of obesity.

VITAMIN A—ESTROGEN INTERACTION ON VAGINAL EPITHELIUM

The keratinizing metaplasia of various mucous epitheliums as the result of vitamin A deficiency has been studied extensively. O. A. Bessey and S. B. Wolbach ("The Vitamins," *p. 54, American Medical Association, Chicago (1939)*) conclude their dis-

cussion of the physiology and pathology of vitamin A with the following statement: "The changes in the repair of epithelium after vitamin A deficiency have their normal counterpart in changes in the vagina of rodents during the part of the estrous cycle in which the cornified vaginal epithelium returns to the mucous type." Various authors have since demonstrated that rather large doses of vitamin A modify or suppress the vaginal smear picture in normal rats or other animals treated with estrogens. These studies have been extended and quantitated in a recent paper by R. H. Kahn (*Am. J. Anat.* **95,** *309 (1954)*).

In order to study the direct effects upon the vaginal epithelium and to work with minimal doses of estrogen, alpha-estradiol and vitamin A dissolved in sesame oil were applied intravaginally using a tuberculin syringe with a blunt needle. Control animals received a like amount of sesame oil alone. All studies were done with mature castrated rats. Vaginal smears were graded on a semi-quantitative scale to determine the effects. Following these experiments, typical animals were killed for histologic study. Sections of the vagina were stained for alkaline phosphatase, polysaccharides and protein-bound sulfhydryl groups.

The application of doses of estrogen ranging from 0.025 to 0.25 I.U. daily for two successive days showed that the technic gave quantitative results. The percentage of positive smears was proportional to the logarithm of the dose of estrogen. A large group of animals was then given 0.10 I.U. of estrogen for six days and daily doses of vitamin A ranging from 0.015 to 1500 I.U. were applied during the last four days of the experiment. The degree of change in the vaginal smear from the second day (prior to vitamin A administration) to the sixth day was shown to be proportional to the logarithm of the dose of vitamin A. Thus vitamin A inhibits or reverses the cornification induced by estrogen in a quantitative fashion dependent upon the dose administered. The smallest dose, 0.015 I.U. daily, produced no significant change, whereas the largest dose caused a reversal to nearly the pre-estrogen smear.

Under the influence of alpha-estradiol the epithelium becomes markedly hyperplastic. The basal cells are cuboidal, the remaining cells become progressively more squamous toward the surface, and the outermost layer becomes keratinized. The total thickness of the epithelium is approximately doubled by the estrogen treatment. When vitamin A was applied simultaneously, the epithelium remained hyperplastic and the total thickness of the epithelium was approximately the same as with estrogen alone, but the surface cells remained nucleated and keratinization was no longer visible. Except for the hypertrophy and hyperplasia, the morphology of the epithelium was similar to that of rats not treated with estrogen. Phosphatase distribution is also similar to that of untreated rats. Sections treated by the periodic acid-Schiff method for polysaccharides showed a marked resemblance to those stained for phosphatase.

Histochemical demonstration of protein-bound sulfhydryl groups in control animals revealed a positive and uniform reaction throughout the stratum basalis, stratum spinosum, and stratum superficialis. When keratinization was present, as in the estrogen-treated animals, the keratinizing cells and the keratin were more reactive. Thus the control and estrogen-treated animals appeared similar except for the intense stain of the stratum corneum in the latter. In the animals treated with estrogen plus vitamin A, only moderate staining was obtained. This was interpreted according to R. J. Barrnett (*J. Nat. Cancer Inst.* **13,** *905 (1953)*) as representing widely separated sulfhydryl groups. Vitamin A treatment apparently decreased the concentration of sulfhydryl groups throughout the epithelium.

These studies demonstrate in quantitative fashion the interrelationships among vita-

min A, estrogen and keratinization of the vaginal epithelium. As low a dose of vitamin A as 0.6 I.U. significantly inhibits the cornification resulting from a total dose of 0.6 I.U. of alpha-estradiol. Both the metaplasia and keratinization are influenced. Which, if either, of these processes represents the primary change induced by estrogen or vitamin A deficiency is of course a matter of speculation since the whole process of cell differentiation is little understood. The authors feel that these studies may indicate a general role of vitamin A in protein anabolism and keratin formation.

DIET AND ENZYME SYNTHESIS

There are numerous reports in the literature, many of them contested, that dietary alterations can evoke changes in the synthesis or secretion of certain digestive enzymes. A high carbohydrate diet fed over a period of time has been found to increase the amylase content of pancreatic juice, while a high protein diet increased the trypsin content. While such adaptation to nutritional conditions might be advantageous to the organism, the effect of diet on enzyme synthesis has not always been found to be in the direction favoring an increase in digestive enzymes. Furthermore, few of these experiments have shed light on the possible mechanism by which diet might influence enzyme formation or secretion. Three recent papers by Tuba and his associates provide additional data as well as considerable fuel for new speculation.

J. Tuba and N. Dickie (*Canad. J. Biochem. Physiol.* **33,** *89 (1955)*) have reported on the effects of various proteins on the alkaline phosphatase content of the intestinal mucosa. Mature male rats were fasted for four days before being offered 5 g. of casein, lactalbumin, or powdered cellulose. Alkaline phosphatase of intestinal homogenates was determined at intervals up to fifteen hours after feeding of the three substances. Cellulose ingestion did not alter the enzyme level from the fasting value. Similarly, lactalbumin produced no change. However, the feeding of casein more than doubled the phosphatase content of the intestine. In a second experiment intestinal alkaline phosphatase was measured six hours after the presentation of the test protein. It was found that lactalbumin, zein, gelatin and wheat gluten did not affect the enzyme level, while the two phosphoproteins, casein and vitellin, caused large and highly significant increases. The authors suggest that alkaline phosphatase is involved in the active transport of amino acids across the intestinal wall, or that at least it is involved in the dephosphorylation of serine phosphate. Evidence for either suggestion is not very strong, and it still remains to be seen what role alkaline phosphatase might play in the digestion of casein and the absorption of its split products. Of equal importance is the question of how the enzyme-synthesizing mechanism adapts to varying dietary situations and responds with such widely varying activity.

Other interesting data which are difficult to interpret were reported by N. Dickie, M. I. Robinson, and J. Tuba (*Canad. J. Biochem. Physiol.* **33,** *83 (1955)*) on the effect of fatty acids on the alkaline phosphatase levels of the intestine. The method of study was similar to that used in the protein experiments already discussed, fasting animals being presented with a single dose of the compound to be investigated. The fatty acids were mixed with a carrier—casein in some experiments, gluten in others—in order to make the material acceptable to the animals. Experiments with the casein carrier showed oleic acid to produce a significant rise in the alkaline phosphatase content of the intestinal wall. This effect was maximal six hours after presentation of

the substance. Stearic acid had little effect. The addition of choline to the casein-oleic acid mixture made the oleic acid much less effective in stimulating enzyme accumulation. When the authors found that the carrier alone caused an increase in enzyme level, additional experiments were designed using wheat gluten as carrier, since this substance was inert. It was found that butyric, lauric, palmitic and oleic acids all stimulated alkaline phosphatase formation or accumulation. It is difficult to accept the authors' contention that there appears to be an inverse relationship between fatty acid chain length and its effect on the phosphatase level. The observed enzyme levels after fatty acid feeding, together with the standard errors, were: butyric acid 7060 ± 532, lauric acid 6730 ± 506, palmitic acid 6780 ± 415. These values are in terms of alkaline phosphatase units per 100 g. of tissue. Stearic acid again gave no significant rise in enzyme concentration. Choline abolished the effect of oleic acid.

It is disturbing that the alkaline phosphatase levels varied so sharply from experiment to experiment. For example, under apparently identical conditions the feeding of casein produced a phosphatase level of 19,500 units per 100 g. in one experiment and a level of 6180 units in another.

In another paper G. S. Wiberg and J. Tuba (*Canad. J. Biochem. Physiol.* **33,** *46 (1955)*) reported on the effect of diet on serum amylase. Adult male rats were maintained on the experimental rations for at least twenty-eight days. The diets were of the synthetic type, varying in the amounts of protein, fat and carbohydrate. The control animals received a commercial stock ration (presumably high in carbohydrate). The synthetic diets rich in either sucrose or starch did not elevate the serum amylase level above that of the control animals receiving a commercial stock ration presumably rich in carbohydrate. A high protein diet (66 per cent casein) significantly lowered the amylase content of serum. On the other hand a fat-rich ration (45 per cent) caused a significant increase in the enzyme level. Studies with diets of various fat levels showed an increase in serum amylase with increasing dietary fat up to 45 per cent, but a depression in enzyme level with 60 per cent fat. A diet free of carbohydrate, one containing cellulose and one containing the poorly digestible raw potato starch caused after some time a depression of serum amylase. A diet of 11 per cent fat and 85 per cent sucrose but no protein significantly lowered the serum enzyme level. A similar mixture of starch and fat caused no significant alteration.

The data presented in these three papers show some clear cases in which enzymatic adaptation to dietary changes functions to improve digestive efficiency. Equally, there are cases where such benefits cannot readily be seen.

THE INHIBITION OF CHOLINE-INDUCED FATTY LIVER BY CORTISONE

Clinical investigators have suggested that cortisone treatment might be beneficial in certain cases of infectious hepatitis and cirrhosis. The therapeutic usefulness of this agent in such conditions cannot be considered to be established as yet, and this hormone is not to be routinely recommended in any clinical disease of the liver. Nevertheless, experimental studies suggest that under certain experimental conditions cortisone may protect the liver from damage.

The experiments of E. A. Sellers and R. W. You (*Biochem. J.* **51,** *573 (1952)*) suggested that the adrenal cortex might play a role in preventing the development of choline deficiency. These workers found that rats fed a low choline diet and simultaneously exposed to cold did not develop

fatty livers. Because adrenal hyperactivity is one of the best known of the physiologic adaptations to cold, its role in preventing choline deficiency was considered. However, Sellers, You, J. H. Ridout, and C. H. Best (*Nature* **166,** *514 (1950)*) were unable to inhibit the development of fatty liver in young rats fed diets low in choline by the administration of 0.5 to 1.0 mg. of cortisone daily. Some relationship between choline metabolism and cortisone was found, however, in that the renal lesions of choline deficiency were inhibited by the hormone.

Evidence supporting the beneficial action of adrenal cortical steroids in choline deficiency has been presented by H. S. Kupperman, E. L. Wyant, and A. C. DeGraff (*Metabolism* **4,** *50 (1955)*). In their experiments immature male rats were fed the choline-deficient diet for periods of seven and fourteen days. In addition to suitable control groups, other groups of rats received either 1 or 2 mg. of cortisone acetate from the start of the diet for periods of seven and fourteen days. One group of rats ate the choline-deficient diet for seven days before receiving a daily injection of 2 mg. of cortisone acetate for the next seven days. After the experimental period, the degree of fatty infiltration was judged by microscopic examination and chemical analysis. Studies in control animals showed that the diet was effective in producing fatty infiltration. The percentage of fat in the liver increased from a normal value of 5.7 to 17.7 after one week on the experimental diet, and to 19.3 after two weeks on the experimental diet. The morphologic changes were comparable to the chemical analyses. The livers of rats receiving cortisone contained much less fat. In the groups receiving 2 mg. a day of cortisone the livers contained 6.5 per cent fat after one week and 7.3 per cent fat after two weeks. The protection exerted by a daily dose of 1 mg. was less complete. After one week of this treatment the percentage of hepatic fat was 11.0 and after two weeks the value was 12.9.

The authors investigated the possibility that a mixture of adrenal cortical steroids, as contained in a commercial cortical extract, might be more active. This was not found to be the case, for after 0.5 ml. of this extract had been given daily to a group of rats for seven days the hepatic fat was 14.8 per cent. A double dose of the cortical extract was actually associated with an increase in hepatic fat. The rise in hepatic fat could not be attributed to the rather large amounts of cottonseed oil administered with the preparation because rats injected with 1 mg. of cortisone plus 1.0 ml. of cottonseed oil daily were found to have 8.4 per cent fat in their livers.

The apparent failure of the cortical extract to protect against the fatty liver of choline deficiency is difficult to explain because by bioassay the dose selected should give an amount of active corticosteroids comparable to that administered to the cortisone group.

It is not apparent in what way the experimental design of Kupperman *et al.* differs from that of Sellers and co-workers, and it is unfortunate that the conflicting results are not discussed by Kupperman *et al.* The mechanism of cortisone action remains to be established. Histochemical evidence of increased glycogen deposition was obtained in the cortisone-treated animals, and the authors suggest that this glycogen might act as a "cushion" against the toxic mechanism responsible for liver disease in man, or protect the liver against damage induced by choline-deficient diets. It should be noted, however, that the dose of cortisone selected was high and might suppress growth. It is known that other experimental procedures which inhibit growth such as caloric restriction (C. H. Best, W. S. Hartroft, C. C. Lucas, and J. H. Ridout, *Brit. Med. J.* **2,** *1001 (1949)*; *Nutrition Reviews* **13,** *186 (1955)*) are known to protect animals against choline deficiency. Under these circumstances the manifestations of defi-

ciency are influenced by the metabolic demands for choline. The finding that cortisone inhibits fatty deposition in an organ will appear to be a paradox to clinicians who are familiar with the peculiar obesity of spontaneous or therapeutic hyperadrenal corticism in man. The failure of cortisone in these experiments to benefit fat already present in the liver argues against any direct clinical application of these observations. The differences between the clinical and experimental diseases seem multiple.

FAT EXCRETION

Normal human subjects generally pass about 5 g. of fat in the feces during each twenty-four hours when they are consuming a diet containing 50 g. of fat daily. This would imply that fat is digested to the extent of about 90 per cent. For the purpose of assessing balance between intake and output of fat, this is a convenient assumption. Fecal fat, however, does not simply represent unabsorbed food fat, but can have at least five different sources, namely: residual dietary fat, bacteria, epithelial debris, carbohydrates and secreted fat. Carbohydrates contribute volatile fatty acids, and fatty acids form up to 5 per cent of the dry matter of intestinal bacteria. Some enteric organisms sythesize fat. Epithelial debris probably contributes to unsaponifiable fecal fat, as could also the bacterial cells. The amount of fat actually excreted from the intestines into the gut is still largely an unknown factor. The extent to which this fraction contributes to the total endogenous fat observed under fat-free dietary conditions is at present not well known.

Apart from its fundamental importance, it is of consequence to obtain some information on endogenous fecal fat in order to define the digestibility of dietary fats more accurately. E. E. Wollaeger, W. O. Lundberg, J. R. Chipault, and H. L. Mason (*Gastroenterology* **24,** *422* (*1953*)) have reported evidence to indicate that in human subjects receiving fat-free diets and then triolein as the only dietary fat, the saturated fatty acids appearing in the feces were derived from saturation of oleic acid by bacteria in the intestine and would therefore not represent endogenous fat primarily.

Detailed studies with rats that have been reported from the University of Minnesota corroborate this view (L. N. Norcia and W. O. Lundberg, *J. Nutrition* **54,** *491* (*1954*)). Two main experiments were performed using 25 rats each. In one experiment weanling male rats were conditioned on a diet containing 15 per cent of tripalmitin as the sole fat, and in the other the animals were conditioned on a diet containing olive oil as the sole fat. Following the thirty-day conditioning period one set of 5 rats from each experiment was killed for fat analyses. Four sets of 5 rats from each of the experiments were placed on ten-day dietary regimens as follows: fat-free diet (dextrose substituted isocalorically for fat); 15 per cent tripalmitin diet; 15 per cent triolein diet; and 15 per cent tripalmitin-triolein diet (1:1 on a weight basis). During the last seven days feces were pooled from each set of animals, and the constituent fats were characterized analytically. Feed intake was closely controlled so that it was virtually the same in the two experiments.

The diets did not support normal growth. In thirty days the tripalmitin-conditioned animals increased their weight about 30 g., whereas the olive oil-conditioned animals gained about 60 g., contrasted with a normal gain of about 100 g. In the case of olive oil-conditioned rats, those subsequently fed triolein excreted about 0.22 g. more fatty acid (0.12 g. of this was monoethenoic acid and 0.09 g. was saturated acid) than the group fed the fat-free diet. The increase in monoethenoic acid was assumed to represent unabsorbed oleate, while the small increase in saturated acid was believed to have

originated from dietary oleate through microbial action. In any event, the differences were small and the results indicated that dietary triolein was virtually completely absorbed, irrespective of conditioning.

Regardless of previous conditioning, rats receiving the fat-free diet and the triolein diet produced feces in which 60 per cent of the total fatty acids analyzed were saturated and about 40 per cent were unsaturated fatty acids (mainly monoethenoic). Qualitatively the fecal lipids were the same in spite of differences in body fat composition induced by previous conditioning diets, a situation that should have influenced fecal fat composition if endogenous fat is a major constituent of fecal fat. Rats consuming tripalmitin excreted forty to sixty times as much fat as rats fed the triolein or the fat-free diet, and about 99 per cent of the fatty acids from feces of tripalmitin-fed animals were saturated fatty acids.

In companion studies Norcia and Lundberg observed that a lard diet increased fatty acid excretion about five-fold over that seen in rats on fat-free diets. This finding, the discovery that rats on a triolein diet (well-absorbed fat) had a fecal fat excretion similar to that of animals on a fat-free diet, plus the finding that animals receiving tripalmitin (poorly absorbed fat) had a very large amount of fecal fat suggest strongly that the fecal fat excretion over and above the endogenous fecal fat is directly related to the absolute amounts of long-chain saturated fatty acids in the diet. This is in agreement with the conclusion of K. F. Mattil (*Oil and Soap* **23,** *344* (*1946*)).

These findings certainly support the view that unabsorbed fat may account for a much larger proportion of the fecal fat than is commonly supposed. An examination of the data regarding unsaponifiable matter in fecal lipids indicated a much higher amount in the tripalmitin-fed rats than in the fat-free and triolein-fed group. An explanation offered is that when dietary fat is poorly absorbed from the bowel, lipids entering the bowel with intestinal juices and glandular secretions would likewise be poorly absorbed.

Conditioning had some influence on the fecal fat in tripalmitin-fed rats. Animals conditioned on tripalmitin, even though smaller in size, absorbed more tripalmitin than the corresponding group previously conditioned on olive oil. This would indicate that the digestibility or absorption of a fat may depend to some extent on the previous dietary history.

An arbitrary measure of the endogenous fecal fat was obtained by summing all fecal fatty acids other than those present in the fat of the diet. Expressed as milligrams per rat per day, these totals were 3.4 and 4.4 for rats fed tripalmitin and fat-free diets, respectively. Values of 4.8 and 5.2 mg. per rat per day were obtained for rats fed triolein and fat-free diets respectively. These results further substantiate the view that the dietary fat has little or no influence on the fecal endogenous fat excretion. They also emphasize again that the total lipid from endogenous sources in the rat is only a small fraction of the total fecal lipid.

Fat deposition in the organs and tissues of the animals in the various groups studied was also investigated (L. N. Norcia and W. O. Lundberg, *J. Nutrition* **54,** *509* (*1954*)). The effect of tripalmitin conditioning in the absence of dietary essential fatty acids was a depletion of fat reserves, a depletion of diethenoic and tetraethenoic acids, and an increase in monoethenoic acids. Thus, in essential fatty acid depletion, the one double-bond fatty acid seems to replace the polyunsaturated fatty acids. This was also observed to occur in the fat of eggs from hens fed fat-free rations (*Nutrition Reviews* **9,** *292* (*1951*)).

The careful studies of Norcia and Lundberg indicate that the composition of endogenous fecal fat is influenced very little by dietary changes in fat and that marked changes in character of stored body fat induced by diet likewise have little influence on endogenous fecal fat. These findings sup-

port the view that endogenous fecal fat is composed primarily of lipids synthesized by intestinal bacteria. Secretion of fat into the intestinal lumen or epithelial debris appears to account for very little of the total endogenous fecal fat in the rat. To this extent the results are in agreement with recent studies on man already mentioned.

THE INFLUENCE OF DIET ON EXPERIMENTAL TUBERCULOSIS

The high incidence of tuberculosis associated in many parts of the world with low standards of living has suggested that diet may have a great deal to do with the resistance to this disease. Investigators have suggested that a number of essential nutrients may be of importance in maintaining resistance. The possible significance of deficiencies of ascorbic acid and vitamin A in this regard have been discussed previously (*Nutrition Reviews* **10,** *141 (1952)*).

In the experimental studies of R. J. Dubos (*J. Exp. Med.* **101,** *59 (1955)*) the objective was to observe the effect of "changes in the physicochemical environment in vivo" on the occurrence of induced tuberculosis in mice. This investigator had previously found that lactate was inhibitory to tubercle bacilli whereas polycarboxylic acids favored the growth of the tubercle bacillus *in vitro*. Experimental procedures were therefore undertaken which might interfere with glycolytic activity of tissue and increase the concentration of acetone bodies and polycarboxylic acids in the environment of the tubercular lesion.

Mice were injected intravenously with strains of pathogenic tubercle bacilli at 29 to 32 days of age. They were then fed a number of experimental diets and their survival was observed.

The effect of limiting food intake was studied with three separate diets. The first was a commercially prepared laboratory chow. The second diet was made up of one-third skim milk and two-thirds wheat flour, and the third diet was a synthetic diet, Sherman no. 191. Acute intermittent undernutrition was induced in mice eating each of these diets by withdrawing the food for thirty hours each week. In each case the mortality due to tuberculosis at periods of from three to seven weeks was greatest in the groups undergoing the periods of acute fasting. The effect seemed to be greatest in the mice on the laboratory chow and least with the synthetic diet. Chronic undernutrition achieved by restriction of the Sherman diet to 3.8 g. per mouse daily had no deleterious effect on the resistance to infection as compared to ad libitum-fed mice. The food restriction amounted to approximately 25 per cent.

It is of considerable interest that, in the experimental system used, protein deficiency had no great detrimental effect on the resistance to mouse tuberculosis. Experimental diets containing between 4 and 20 per cent skim milk as the source of protein did not alter the rate of death due to induced tuberculosis provided that fat was incorporated in the diet in the form of either peanut oil or cocoa butter. Mortality was accelerated by the low protein diet in the absence of fat.

In a large number of experiments 8 to 10 per cent citrate was added to experimental diets containing from 4 to 19 per cent skim milk and differing in their fat content. There was an acceleration of mortality among those mice receiving citrate. In a similar fashion the addition of 2 per cent sodium citrate or 1 per cent sodium glutarate to the drinking water decreased the period of survival whereas alcohol or normal saline solution was without such effect.

The addition of thyroid extract or dinitrophenol to the diet or drinking water in most cases decreased the survival time. The fact that resistance to tuberculosis had been altered with dinitrophenol and citrate was demonstrated in another manner by experiments with the BCG strain of the tubercle

bacillus. This organism is nonpathogenic in normal mice, but in mice given either dinitrophenol or citrate a slowly progressive fatal disease developed.

The simple test system used by Dubos permits the rapid screening of many factors which might alter the resistance of the host to the tubercle bacillus and it is obvious in this report that a number of dietary and other factors have been demonstrated to decrease resistance. Certain shortcomings of the design of the experiments might be suggested. The use of ad libitum diets complicates the interpretation. For example, the weight loss which occurred in mice receiving dinitrophenol in their diet cannot be attributed to hypermetabolism until the factor of decreased food intake due to unpalatability can be ruled out. In the experiments in which sodium citrate is incorporated into the diet it might be legitimate to wonder whether the effect might be attributable to the potential alkali which is ingested rather than due to the citrate per se.

The mechanism by which resistance to tubercle bacilli was reduced in these experiments may have nothing to do with simple alterations in the glycolytic cycle. One of the most intriguing observations of this paper is the deleterious effect of repeated short periods of starvation in contrast to the lack of effect of chronic undernutrition. It is known that complete starvation may activate the pituitary-adrenal cortical system whereas chronic undernutrition decreases adrenal activity (*Nutrition Reviews* **11,** *156 (1953)*). In view of the known effect of adrenal steroids on decreasing resistance to experimental and clinical tuberculosis the suggestion might be made that the adrenal was responsible for the more rapid course of the disease in the mice subjected to repeated short periods of starvation.

The importance of the problem which Dubos has set out to investigate should be apparent to all readers of this exploratory paper. The problems of host resistance have not all been answered by the technics of immunology. The suggestion of Dubos that relatively simple biochemical changes in the neighborhood of the infective agent may influence the balance between parasite and host deserves more investigation.

LIPOTROPIC ACTIVITY OF PROTEINS

Since the discovery by Best and his associates that choline could prevent or reverse excessive accumulation of lipids in the liver, much attention has been given to the role of protein in studies of lipotropic activity. The level of dietary protein was found to have a profound effect on liver fat in choline deficiency (C. H. Best and H. J. Channon, *Biochem. J.* **29,** *2651 (1935)*). At the same time it became apparent that proteins differed widely in their ability to prevent fatty livers (Channon *et al., Ibid.* **32,** *976 (1938)*). H. F. Tucker and H. C. Eckstein (*J. Biol. Chem.* **126,** *117 (1938)*) attributed these differences among proteins to their differing methionine content. Cystine was found to possess considerable antilipotropic activity. Casein showed a distinctly stronger lipotropic action than a mixture of methionine and cystine in the amounts present in the casein supplement. This suggested strongly that factors in proteins other than methionine and cystine were involved in determining the lipotropic activity of the protein.

C. C. Lucas and J. H. Ridout (*Canad. J. Biochem. Physiol.* **33,** *25 (1955)*) have reinvestigated the lipotropic activity of proteins since, in their stated opinion, all previous investigations on the subject involved several uncontrolled variables. The test diets employed contained 3, 6, 9, or 18 per cent casein with enough methionine added to bring the methionine content of the diets to a total of 0.54 per cent (the level of methionine in the 18 per cent casein diet). All

diets contained in addition 0.12 per cent choline chloride. Thus the level of choline and its precursor, methionine, was kept at a constant level in all four diets. The only differences among the diets was in the amount of amino acids other than methionine and in the somewhat lower carbohydrate content of the diets containing the larger amounts of protein. The experimental animals, young male rats, received the rations ad libitum.

With 3 per cent casein in the diet the animals showed no gain in weight during the three-week experimental period. The liver fat level was about 16 per cent. At the 6 per cent casein level liver fat was reduced to 11 per cent. At the 9 per cent protein level liver fat was 9 per cent, and with 18 per cent casein it was reduced to 7 per cent. Growth increased progressively with increasing dietary protein. Food consumption on the 18 per cent casein diet was nearly three times that with the 3 per cent protein level, showing the difficulty of restricting the experiment to a single variable.

A distinct lipotropic effect of casein was observed in the absence of choline as well as in the presence of 0.36 per cent choline (methionine being kept constant at a level of 0.54 per cent in all diets). The lipotropic action was, however, less pronounced under these conditions.

The distribution of lipids in livers of animals receiving the low protein diets differed from that seen in choline deficiency. In protein deficiency the fat is concentrated at the periphery of the liver lobule. In choline deficiency the fat appears first and most heavily about the central vein.

The authors point out that threonine as well as other dietary factors have been shown to influence the level of liver fat. They also look favorably upon the suggestion made by F. Dick and co-workers (*Arch. Path.* **53,** *154 (1952)*) that some fatty livers may result from nonspecific effects of protein deficiency rather than from the lack of some specific amino acids. They point out that protein contributes in three ways to the protection of the liver: (1) by furnishing methionine as a methyl donor for choline synthesis, (2) by exerting a lipotropic effect independent of methionine, and (3) by maintaining the architectural integrity of the liver cells. The authors also appear to lean toward the idea that some of the lipotropic activity of proteins can be explained in terms of the effective synthesis of lipoprotein complexes.

SULFUR AMINO ACIDS AND THE REGENERATION OF WOUND TISSUE

The nutritional state of the animal as well as the immediate effect of diet have long been known to influence the speed and degree of recovery from wounds. Protein is required not only for the formation of new tissue but also to compensate for the loss of nitrogen associated with the initial trauma. It has been observed that methionine and cystine appear to have special roles in effecting early and complete recovery from experimental wounding (*Nutrition Reviews* **6,** *274 (1948)*; **11,** *124 (1953)*). It was thought that these two amino acids must act chiefly through an effect on synthesis of regenerating tissue protein and/or on sulfhydryl enzymes involved in the recovery process.

Through the use of tracer technics and analysis of wound and other tissues for cystine and methionine, M. B. Williamson and H. J. Fromm (*J. Biol. Chem.* **212,** *705 (1955)*) have contributed further information on this problem. For this study, female albino rats weighing 200 g. were used. During a five-day protein depletion period all rats were maintained on a protein-free synthetic diet which was adequate in other respects. On the sixth day the hair was removed from the back and shoulders, the rats were anesthetized, and a circular wound

4 cm. in diameter was made by excising the tissue down to the fascia. The tissue was then removed with the underlying fascia.

In the first experiment the rats were wounded and placed on the basal diet supplemented with 6 per cent casein. The total protein content of the newly formed wound tissue rose gradually until the twentieth day, when it reached a normal level. The methionine concentration rose at about the same rate as the protein and reached a plateau at a level about one-third higher than normal tissue on the twenty-third day. The cystine content, on the other hand, rose more rapidly and was maintained at a relatively higher level than methionine. Thirty-one days after wounding the methionine content was 6.3 mg. per gram of wet tissue, whereas the content of normal skin was 4.6 mg. Similarly, the cystine content was elevated at 8.2 mg. per gram as compared with a normal value of 3.0 mg. Thirty-one days after wounding it was impossible to distinguish accurately regenerated wound tissue from normal tissue by visual inspection. Therefore, it is not known whether these values would eventually have returned to normal, or how long this would have taken if the decline had occurred.

In the second experiment 30 rats were placed on the basal diet supplemented with 0.1 per cent cystine. Half of these animals were wounded and the other half served as controls. All rats received by intraperitoneal injection a tracer dose of S^{35}-labeled DL-cystine five days after the time of wounding. Animals were killed for analysis at seven, eleven and fourteen days after wounding. The methionine, cystine and nitrogen contents of the wound tissue followed essentially the same pattern as described above. The livers of the wounded rats differed from the controls in that the methionine content, which was normal at the seventh day, dropped from 6.5 to 4.6 mg. per gram of wet tissue between the seventh and the fourteenth days. This suggests that methionine was probably being converted to cystine to meet the high demand for that amino acid.

On the seventh day after wounding, the S^{35} activity was found to be highest in the liver and wound tissue, with smaller quantities in the skin and muscle. Between the seventh and fourteenth days the S^{35} activity of wound tissue dropped 23 per cent, skin 52 per cent, muscle 5 per cent, and liver 55 per cent. Loss of the labeled sulfur from the skin and liver of control rats was somewhat lower; however, these rats showed a 22 per cent loss of S^{35} in the muscle as compared with the 5 per cent loss in the wounded rats.

A third experiment was similar to the second except that the basal diet was fed unsupplemented and a single dose of DL-methionine-S^{35} was administered four days after the time of wounding. Animals were killed for analysis at five, nine and thirteen days after wounding. The radioactive sulfur was about equally distributed between methionine and cystine in the livers of wounded as well as control rats. In wound tissue the concentration of S^{35} in cystine was about three-fourths that in methionine. In both groups of rats the loss of labeled cystine from the liver between the fifth and thirteenth days was 40 per cent. The loss of labeled methionine from the livers of wounded rats was twice that of control animals. The methionine-S^{35} of wound tissue decreased 62 per cent between the fifth and thirteenth days, whereas the decrease of cystine-S^{35} was only 6 per cent. These data demonstrate the efficient conversion of methionine to cystine under these conditions, as well as the relatively greater conversion in wounded rats. It would be interesting to know whether the large amount of methionine-S^{35} lost from the wound tissue was appreciably re-utilized through conversion to cystine.

The rate at which radioactive sulfur was excreted via the urine would have aided in interpretation of these data. In a previous paper, M. B. Williamson and H. J. Fromm

(*Proc. Soc. Exp. Biol. Med.* **87,** *366 (1954)*) reported a greater urinary excretion of radioactive sulfur following cystine-S^{35} administration than after methionine-S^{35}.

No mention was made in this paper (*J. Biol. Chem., loc. cit.*) regarding the effect of wounding upon weight of the animals or upon food consumption during the experimental period. If wounding did result in loss of weight and alteration of dietary intake, these factors may have influenced the results of this study. In that case, use of pair-fed control animals would have added greatly to the value of the data. The effect of cystine and methionine in aiding the healing of wounds is of practical importance as well as of basic nutritional significance. This effect appears to be due largely to the high content of the amino acids in the wound tissue.

POLYOXYETHYLENE STEARATES IN FOOD

The Food Protection Committee of the Food and Nutrition Board has made a thorough evaluation of the evidence relating to the safety of the polyoxyethylene stearates as food additives (*National Academy of Sciences—National Research Council, Publication No. 280, July 1953*). The Committee concludes in part that, "The introduction into a foodstuff of a new additive which does not positively contribute to the nutritional quality of the food presents a situation demanding particularly conservative judgment," and that, "this Committee can only conclude that the available data fail to demonstrate that polyoxyethylene stearates are safe for use in foods under all patterns of dietary consumption . . ." The report does not condemn these materials as additives, but concludes that the amount of evidence is insufficient at present to declare them safe. Thus new data, such as those presented by W. A. Krehl, G. R. Cowgill, and A. D. Whedon (*J. Nutrition* **55,** *35 (1955)*) are of particular interest.

The experiments consisted of feeding high levels of polyoxyethylene (POE) compounds to rats and cats, measurement of rate of growth and food consumption, examination of size of the liver, kidneys, adrenals and testes at autopsy, and study of the tissues for gross or microscopic pathology. In the rat studies eleven groups of 9 animals each were used in nearly all cases. The diets were of the familiar purified type but somewhat higher in fat than levels ordinarily used. The C-1 diet contained 5 per cent corn oil and 12 per cent lard while the C-2 diet contained 5 per cent corn oil and 18 per cent lard. In the C-1 diet, 6 per cent of each of the following materials was substituted for an equivalent amount of lard: POE monostearate, mono-oleate, dilaurate, distearate, monolaurate and dioleate, as well as the POE alone. Using the C-2 diet containing 18 per cent lard, one group fed a diet containing 10 per cent of the monostearate was compared to the control. The animals were followed for approximately 500 days.

In the cat experiments a 30 per cent fat diet was used. The 6 experimental cats received 20 per cent of the polyoxyethylene monostearate in place of an equivalent amount of lard. Six control animals were used. The animals were young and weighed a little over a kilogram when the studies started. They were killed for autopsy after eight months.

The results of these studies can be simply summarized. No effect on growth or survival time was observed, and no pathologic changes that could be ascribed to the bread softeners were seen. Somewhat more food was eaten by the experimental groups than by the controls, and this might be expected since the polyoxyethylene part of the bread softeners is not utilizable. One cat refused to eat the diet containing 20 per cent of the monostearate.

The gross and histopathologic changes seen in the rats were those ordinarily seen in old rats. The most frequent abnormalities were congestion of the lungs and casts in the kidneys. Numerous other changes were described in occasional animals, but there was no evidence that they were caused by or related to the diets used. Similarly, the cats were all normal by gross inspection. Microscopic examination of the tissues of the control animals showed minor changes in the liver of one, the spleen of one, and the thyroid of 3. Two showed some nephritis. In the experimental animals one animal showed some hyperplasia of the reticulum of the spleen. This was the only change reported.

As the authors are careful to point out, it is generally unwise to make sweeping conclusions applicable to man upon the basis of animal experiments. On the other hand, they provide one of the few guides available to evaluate probable effects on human beings and they have proven extremely valuable in the past. The levels of intake achieved in these studies exceed many times those that might be reasonably expected in practical human nutrition. The Food Protection Committee of the Food and Nutrition Board (*loc. cit.*) estimated that a diet containing substantial amounts of bread, rolls, doughnuts, and ice cream which contained Myrj 45 (polyoxyethylene-8-monostearate) in the amounts commonly used might supply this compound at approximately the 0.1 per cent level. Under unusual circumstances it was estimated that it might go as high as 0.6 per cent. "For example, on a dry weight basis, a 2 per cent diet for the rat compared with 0.1 per cent for man would represent a safety to 20 to 1." The levels studied here of 6, 10 and 20 per cent in the diet would represent comparable safety factors of 60, 100 and 200. Actually, of course, this is the most conservative means of calculating the safety margin. Since the small animals consume more food per unit of body weight than man, the difference in intake per unit weight would be even greater than the ratios presented.

From the results obtained, "no reasonable doubt remains in the authors' minds concerning the safety of the polyoxyethylene stearates when fed in quantities constituting up to 20 per cent of the diet under the conditions of the experiments." Certainly most readers will agree that this work constitutes strong evidence of the safety of these materials as food additives. The point that critics will be quick to pick up is the qualifying phrase "under the conditions of the experiment." Whether the type of diet used in the studies is a significant factor in their outcome is unknown, but, as the Food Protection Committee has emphasized, the few reports which have indicated some untoward effect are those which must be considered seriously until some explanation of the cause is apparent.

THE ANTITHYROTOXIC FACTOR

Hyperthyroidism induced in experimental animals causes an increase in the requirement of a number of nutritional factors as well as calories. The possibility that the metabolic stress of hyperthyroidism may induce deficiency of hitherto unrecognized nutrients has been suggested. The experiments of B. H. Ershoff bearing on this problem have been discussed previously (*see Nutrition Reviews* **11,** *276 (1953)*; **13,** *33 (1955)*). This worker found that the addition of desiccated thyroid to a purified diet containing no fat resulted in decreased growth and increased mortality in rats. Supplements of known B-vitamins did not restore growth of rats fed a fat-free diet but were partially effective in restoring growth of rats fed 2 per cent fat. With diets containing 10 per cent fat the rate of growth was nearly normal. A water-insoluble liver residue was

successful in maintaining growth at all levels of dietary fat.

Progress in isolating and defining the principle which promotes the growth of hyperthyroid rats has been reported by B. L. O'Dell, S. J. Stolzenberg, J. H. Bruemmer, and A. G. Hogan (*Arch. Biochem. Biophys.* **54,** *232 (1955)*). The basal diet used in these experiments contained 22 per cent casein as the protein source. Fat was provided as lard, comprising 5 per cent of the diet. The diet was also supplemented with the vitamins known to be required by the rat. Hyperthyroidism was induced by addition of 0.15 per cent iodinated casein. Antithyrotoxic activity was assayed by comparing the rate of growth of groups of rats receiving the basal diet, the basal diet plus 10 per cent of liver residue, and the basal diet supplemented with the test substance. The results of the assays were only roughly quantitative.

Other sources of antithyrotoxic factor were sought without much success. Some activity was found in the aqueous extracts of liver, but this was less than that in the insoluble liver residue. Brewers' yeast was inactive when incorporated in amounts of 10 per cent of the diet. Moderate activity was found in wheat germ and soybean oil meal. An aqueous extract of soybean oil was inactive at a level of 5 per cent of the diet. The insoluble residue of soybean oil meal was roughly half as active as the reference liver residue. Gelatin substituted for casein in an amount of 10 per cent of the total diet did not promote growth.

A number of technics for making the antithyrotoxic factor in the liver residues more soluble were studied. Enzymatic digestion of the liver residue with pepsin, pancreatin and trypsin seemed to release the activity. This also could be achieved by autoclaving in the presence of either sodium hydroxide or sulfuric acid. The last procedure appeared to be the most promising.

No chemical data are presented concerning the properties of the antithyrotoxic factor. Negative assays were obtained with lyxoflavin, thioctic acid, orotic acid, and guanine. A mixture of glycine, methionine and L-arginine was without effect. Supplementation with magnesium and potassium was also unsuccessful.

The effects of molybdenum and liver residue on the xanthine oxidase of the rat intestine were compared. Both agents caused an eight-fold increase in the xanthine oxidase activity in nonhyperthyroid rats. In the thyrotoxic rats, liver residue induced the same degree of xanthine oxidase increase as before, whereas molybdenum only increased the enzyme activity to two thirds of that observed in the control group receiving molybdenum. Molybdenum did not stimulate growth in the thyrotoxic rats.

The isolation of the antithyrotoxic factor, if there should be such a factor, is greatly hampered by the laborious and inexact assay which is available. The results reviewed continue to suggest the existence of such a nutrient, although its ultimate significance remains unclear.

ERRATUM

Volume 13, page 147, column two, paragraph one, line one should read:

"The requirement of rats for thiamine has been placed at 2000 micrograms of thiamine. . ."

NOTES

Letter to the Editor

Dear Sir:

In view of the conclusions that your reviewer has drawn from the report on vitamin supplementation of Army personnel and physical performance in the cold (*Nutrition Reviews* **13,** *102 (1955)*), I as one of the advisers on this experiment would like to make the following comments:

1. There was no adequate control data on the men in their normal environment.

2. There was no real period of stabilization at Pole Mountain prior to initiating the supplementation.

3. The supplemented group did show improvement in several respects.

In view of these facts, it seems to me that the data did not rule out the possible role of certain nutritional fractions in increasing the capacity to withstand *cold* stress which was the original purpose of this experiment. This latter purpose was never accomplished because the winter at Pole Mountain was the warmest in eighty years and hence the men were not sufficiently exposed to cold.

Unhappily, this experiment proved, in my opinion, nothing.

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Ascorbic Acid and Mercurial Diuresis

Ascorbic acid is sometimes administered with mercurial diuretics, because in experimental animals it protects against cardiac arrest which is one of the toxic effects of these agents. As it has been assumed that the ascorbic acid acts as a reducing agent in lowering the toxicity of the mercurial, it seemed desirable to investigate the effect of ascorbic acid upon the diuretic activities as well as on the toxicity. This has been done by H. S. Tacket and M. L. Gubin (*J. Clin. Invest.* **33,** *844 (1954)*).

The results indicate that the depression in the maximum tubular excretion of para-aminohippurate effected by mercuhydrin is blocked by ascorbic acid (150 mg. doses, given intravenously). This suggests that the diuretic effect of mercuhydrin might be reduced by the administration of ascorbic acid, as a reduction in tubular reabsorption is presumably the important mechanism of mercurial action.

The authors are rightfully conservative, however, in indicating that their studies only indicate the relation of ascorbic acid to the para-aminohippurate tubular maximum, and merely suggest that these sizable doses of ascorbic acid may interfere with the action of the mercurial as a diuretic. The effect of the ascorbic acid was similar in quality and degree to that of British antilewisite (BAL).

Nutrition Issue

The March 1955 number of *Postgraduate Medicine* (the Official Journal of the Interstate Postgraduate Medical Association, Publishers, Essex Building, Minneapolis 3, Minnesota) has been prepared, in large part, by Dr. Tom Spies, in collaboration with a number of his associates in this country as well as associates in Puerto Rico and Cuba. Most of this issue deals with their report on case histories of acute nutritional deficiencies that they have encountered in studies in recent years. An excellent and large collection of colored photographs is included, which should prove very useful in teaching.

CLINICAL NUTRITION BRIEFS

"The primary importance of the observations of Lear and his associates would seem to reside in the fact that they offer further confirmation of the nature of pernicious anemia as a deficiency disease. This concept is supported by the absence of detectable free vitamin B_{12} and the low total vitamin B_{12} concentration of serum of patients with this type of anemia and by the restoration of these levels to normal with vitamin B_{12} treatment." (*Nutrition Reviews* **13,** *100* (*1955*), Vitamin B_{12} Concentration in Serum of Patients with Pernicious Anemia.)

"The above studies failed to demonstrate that the addition of large doses of B-vitamins and of ascorbic acid to a ration adequate enough to maintain level of performance and to prevent clinical nutritional deficiency states in soldiers undergoing physical activity in the cold improves their physical or mental performance. Until convincing evidence is presented to the contrary, it seems reasonable to assume that maximal physical performance can be expected of industrial workers or of soldiers if they are provided with adequate diets consisting of natural foodstuffs." (*Nutrition Reviews* **13,** *102* (*1955*), Vitamin Supplementation and Physical Performance.)

"The association of intracellular accumulation of fat in the livers of patients convalescing from or with persisting hepatitis has not been described by other authors. Therefore, the observations described by Colwell are original and new." (*Nutrition Reviews* **13,** *105* (*1955*), Excess Weight Gain and Fatty Liver in Viral Hepatitis.)

"Although the 15 wounded men had lower than normal serum albumin values (range 2.0 to 4.4 g. per cent, average 3.3) compared to the control group (average 4.3 g. per cent), there was no apparent relationship between the capacity to produce antibody and serum albumin, globulin, or total serum protein level. Also, the age of the patient, clinical condition, or degree of malnutrition did not appear to be related to the antibody response." (*Nutrition Reviews* **13,** *108* (*1955*), Malnutrition and Antibody Formation.)

"These data strongly suggest that the stannous chlorofluoride solution was a more effective agent for the topical treatment of tooth surfaces than sodium fluoride. Indeed, its effectiveness in this one-year period is comparable to the amount of reduction that is observed after the ingestion of fluoridated water during tooth development." (*Nutrition Review* **13,** *134* (*1955*), Stannous and Fluoride Compounds for Topical Dental Treatments.)

"A relation between diet, serum cholesterol and atherosclerosis is well recognized. Available evidence points to the fat content of the diet as an important factor in the relationship." (*Nutrition Reviews* **13,** *138* (*1955*), Diet, Cholesterol and Atherosclerosis.)

"It is abundantly clear from the data presented that no difference exists in the availability from enriched bread of the four radioactive iron preparations which were studied . . . It is clearly demonstrated that these patients were able to absorb iron quite efficiently from the compounds in enriched bread." (*Nutrition Reviews* **13,** *165* (*1955*), Utilization of Iron Compounds in Enriched Bread.)

"The over-all dietary records show that boys eat more than girls. At equal weights the intake of the boys appears to be about 10 per cent higher than that of the girls. Calorie intakes for both boys and girls were considerably above National Research Council Allowances for those between 6 and 8 years of age, but after 10 years they agreed well with the Recommended Allowances." (*Nutrition Reviews* **13,** *167* (*1955*), Nutritional Status of Iowa School Children.)

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